
Sequence Listing was accepted.

If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).

Reviewer: Durreshwar Anjum

Timestamp: [year=2008; month=1; day=18; hr=14; min=41; sec=8; ms=290;]

Validated By CRFValidator v 1.0.3

Application No: 10049358 Version No: 4.0

Input Set:

Output Set:

Started: 2008-01-07 14:17:01.771 **Finished:** 2008-01-07 14:17:03.067

Elapsed: 0 hr(s) 0 min(s) 1 sec(s) 296 ms

Total Warnings: 13
Total Errors: 0

No. of SeqIDs Defined: 16

Actual SeqID Count: 16

Error code		Error Descripti	ion								
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(4)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(5)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(6)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(7)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(8)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(9)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(10)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(11)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(12)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(13)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(14)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(15)
W	213	Artificial	or	Unknown	found	in	<213>	in	SEQ	ID	(16)

SEQUENCE LISTING

<110> DAVIS, Maria NELSON, John KUMAR, Shiv FINN, Patrick NAMPALLI, Satyam FLICK, Parke

<120> TAQ DNA POLYMERASES HAVING AN AMINO ACID SUBSTITUTION AT E681 AND HOMOLOGS THEREOF EXHIBITING IMPROVED SALT TOLERANCE

<130> PB9944

<140> 10049358

<141> 2002-05-17

<150> PCT/US00/22150

<151> 2000-08-10

<150> US 60/154,739

<151> 1999-09-17

<150> US 60/150,167

<151> 1999-08-21

<160> 16

<170> PatentIn version 3.3

<210> 1

<211> 832

<212> PRT

<213> Thermus aquaticus

<400> 1

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu 1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80

Tyr	Lys	Ala	Gly	Arg 85	Ala	Pro	Thr	Pro	Glu 90	Asp	Phe	Pro	Arg	Gln 95	Leu
Ala	Leu	Ile	Lys 100	Glu	Leu	Val	Asp	Leu 105	Leu	Gly	Leu	Ala	Arg 110	Leu	Glu
Val	Pro	Gly 115	Tyr	Glu	Ala	Asp	Asp 120	Val	Leu	Ala	Ser	Leu 125	Ala	Lys	Lys
Ala	Glu 130	Lys	Glu	Gly	Tyr	Glu 135	Val	Arg	Ile	Leu	Thr 140	Ala	Asp	Lys	Asp
Leu 145	Tyr	Gln	Leu	Leu	Ser 150	Asp	Arg	Ile	His	Val 155	Leu	His	Pro	Glu	Gly 160
Tyr	Leu	Ile	Thr	Pro 165	Ala	Trp	Leu	Trp	Glu 170	Lys	Tyr	Gly	Leu	Arg 175	Pro
Asp	Gln	Trp	Ala 180	Asp	Tyr	Arg	Ala	Leu 185	Thr	Gly	Asp	Glu	Ser 190	Asp	Asn
Leu	Pro	Gly 195	Val	Lys	Gly	Ile	Gly 200	Glu	Lys	Thr	Ala	Arg 205	Lys	Leu	Leu
Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
Asp	Phe	Ala	Lys 260	Arg	Arg	Glu	Pro	Asp 265	Arg	Glu	Arg	Leu	Arg 270	Ala	Phe
Leu	Glu	Arg 275	Leu	Glu	Phe	Gly	Ser 280	Leu	Leu	His	Glu	Phe 285	Gly	Leu	Leu
Glu	Ser 290	Pro	Lys	Ala	Leu	Glu 295	Glu	Ala	Pro	Trp	Pro 300	Pro	Pro	Glu	Gly

Ala P1 305	he Val	Gly	Phe	Val 310	Leu	Ser	Arg	Lys	Glu 315	Pro	Met	Trp	Ala	Asp 320
Leu L	eu Ala	Leu	Ala 325	Ala	Ala	Arg	Gly	Gly 330	Arg	Val	His	Arg	Ala 335	Pro
Glu P	ro Tyr	Lys 340	Ala	Leu	Arg	Asp	Leu 345	Lys	Glu	Ala	Arg	Gly 350	Leu	Leu
	ys Asp 355					360					365			
3	ly Asp 70	-			375			-		380	-			
385	hr Pro			390					395					400
	la Gly ly Arg		405					410					415	
_	lu Arg	420					425					430	_	
	435 rg Leu					440					445			
	50 lu Ile	Ala	Arg	Leu	455 Glu	Ala	Glu	Val	Phe	460 Arg	Leu	Ala	Gly	His
465 Pro P	he Asn	Leu	Asn	470 Ser	Arg	Asp	Gln	Leu	475 Glu	Arg	Val	Leu	Phe	480 Asp
Glu L	eu Gly	Leu	485 Pro	Ala	Ile	Gly	Lys	490 Thr	Glu	Lys	Thr	Gly	495 Lys	Arg
Ser T	hr Ser	500 Ala	Ala	Val	Leu	Glu	505 Ala	Leu	Arg	Glu	Ala	510 His	Pro	Ile
	515					520			-		525			

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr

530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu 545 550 555 560 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 580 585 590 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr 625 630 635 640 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro 645 650 655 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 660 665 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu 675 685 680 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 690 695 700 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Gly Tyr Val 705 710 715 Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 725 730 735 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro 745 740

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu

765

760

755

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His 775 780 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 <210> 2 <211> 561 <212> PRT <213> Thermus aquaticus <400> 2 Met Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu 1 5 10 Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Glu 20 25 30 Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala 35 40 45 Asp Leu Leu Ala Leu Ala Ala Arg Gly Gly Arg Val His Arg Ala 50 55 60 Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu 65 70 75 80 Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu 90 85 Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser 100 105 110

Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr

115 120 125

Glu	Glu 130	Ala	Gly	Glu	Arg	Ala 135	Ala	Leu	Ser	Glu	Arg 140	Leu	Phe	Ala	Asn
Leu 145	Trp	Gly	Arg	Leu	Glu 150	Gly	Glu	Glu	Arg	Leu 155	Leu	Trp	Leu	Tyr	Arg 160
Glu	Val	Glu	Arg	Pro 165	Leu	Ser	Ala	Val	Leu 170	Ala	His	Met	Glu	Ala 175	Thr
Gly	Val	Arg	Leu 180	Asp	Val	Ala	Tyr	Leu 185	Arg	Ala	Leu	Ser	Leu 190	Glu	Val
		195					200					205		Ala	_
	210					215					220			Leu	
225					230				_	235				Gly	240
-				245					250					255 Lys	
			260				_	265					270	Gly	
		275					280					285		Leu	
Ser	290 Ser	Asp	Pro	Asn	Leu	295 Gln	Asn	Ile	Pro	Val	300 Arg	Thr	Pro	Leu	Gly
305 Gln	Arg	Ile	Arg	Arg	310 Ala	Phe	Ile	Ala	Glu	315 Glu	Gly	Trp	Leu	Leu	320 Val
Ala	Leu	Asp	Tyr	325 Ser	Gln	Ile	Glu	Leu	330 Arg	Val	Leu	Ala	His	335 Leu	Ser
		-	340					345	-				350		

Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His

355 360 365

Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp 370 375 380

Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Tyr Gly Val Leu Tyr 385 390 395 400

Gly Met Ser Ala His Arg Leu Ser Gln Arg Leu Ala Ile Pro Tyr Glu \$405\$ \$410\$ \$415

Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val $420 \hspace{1.5cm} 425 \hspace{1.5cm} 430$

Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr 435 440 445

Val Glu Thr Leu Phe Gly Arg Arg Tyr Val Pro Asp Leu Glu Ala 450 455 460

Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met 465 470 475 488

Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys 485 490 495

Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val 500 505 510

His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val 515 520 525

Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val $530 \hspace{1.5cm} 535 \hspace{1.5cm} 540 \hspace{1.5cm}$

Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys 545 550 555 560

Glu

<210> 3 <211> 832 <212> PRT

<213> Thermus aquaticus

<400> 3

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu $1 \ \ \, 5 \ \ \, 10 \ \ \, 15$

Val Ala Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly 20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala 35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val 50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly 65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu 85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu 100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys 115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp $130 \,$ $135 \,$ $140 \,$

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro 165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn 180 185 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu 195 200 205

Glu	Glu 210	Trp	Gly	Ser	Leu	Glu 215	Ala	Leu	Leu	Lys	Asn 220	Leu	Asp	Arg	Leu
Lys 225	Pro	Ala	Ile	Arg	Glu 230	Lys	Ile	Leu	Ala	His 235	Met	Asp	Asp	Leu	Lys 240
Leu	Ser	Trp	Asp	Leu 245	Ala	Lys	Val	Arg	Thr 250	Asp	Leu	Pro	Leu	Glu 255	Val
			260					265					270	Ala	
		275					280					285		Leu	
	290		-			295					300			Glu	
305					310				_	315				Ala	320
				325					330					335 Leu	
			340					345					350	Leu	
Pro	Gly	355 Asp	Asp	Pro	Met	Leu	360 Leu	Ala	Tyr	Leu	Leu	365 Asp	Pro	Ser	Asn
Thr	370 Thr	Pro	Glu	Gly	Val	375 Ala	Arg	Arg	Tyr	Gly	380 Gly	Glu	Trp	Thr	Glu
385 Glu	Ala	Gly	Glu	Arg	390 Ala	Ala	Leu	Ser	Glu	395 Arg	Leu	Phe	Ala	Asn	400 Leu
Trp	Gly	Arg	Leu	405 Glu	Gly	Glu	Glu	Arg	410 Leu	Leu	Trp	Leu	Tyr	415 Arg	Glu
			420					425					430		

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly

440 445 435

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala 450 455 460 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His 470 475 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp 485 490 495 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg 500 505 510 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile 515 520 525 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr 530 535 540 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu 545 550 555 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 565 570 575 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 585 580 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala 595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly 610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gl